



# HOKKAIDO UNIVERSITY

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| Title               | Development of novel methods for rapid and efficient extraction of naturally occurring bioactive sphingoid bases [an abstract of dissertation and a summary of dissertation review] |
| Author(s)           | SIDDABASAVEGOWDA, B.                                                                                                                                                                |
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学 位 論 文 審 査 の 要 旨  
Doctoral Dissertation Evaluation Review

博士の専攻分野の名称 博士（生命科学）

氏 名 SIDDABASAVEGOWDA B

Degree requested: Doctor of Life Science

Name: SIDDABASAVEGOWDA B

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|           |                         |       | (先端生命科学研究院/Faculty of Advanced Life Science) |                                   |

学 位 論 文 題 名

Title of Doctoral Dissertation

“Development of novel methods for rapid and efficient extraction of naturally occurring  
bioactive sphingoid bases”

(生理活性を有する天然由来スフィンゴイド塩基類の迅速かつ効率的な新規抽出法の開発)

博士学位論文審査等の結果について（報告）

Results of Evaluation of the Doctoral Dissertation (Report)

Recently, studies on bioactivities of natural sphingoid bases are actively being done. However, methods for efficient extraction/preparation of those sphingoid bases were limited due to their low abundance and difficulty in purification.

In this study, due to the limitations of current extraction methods for sphingoid bases, the author performed a novel research, on developing two novel methods for extraction/preparation of sphingoid bases. The established methods were superior to conventional extraction methods in its rapidity, efficiency and cost effectiveness. These methodologies are crucial requirement for the discovery of a novel sphingoid bases from natural resources and to evaluate their biological activity. Structurally, all sphingoid bases posses a common 2-amino 1,3 diol moeity, and these functional group known to react chemoselectively with glutaraldehyde at room temperature. Based on these key idea, the author developd a novel glutaraldehyde resin (GR) based rapid and facile extraction method for chemoselective capture of sphingoid bases from biological samples. The GR was synthesized in gram-scale starting from commercially vailable *trans-p*-coumaric acid in eight steps and its stability was confirmed from IR-spectroscopy. The results revealed that the GR was successfully applied for the enrichment of endogeneous sphingoid bases from human seum (sphingosine) and golden oyster fungi (9-methyl sphingadienine and glucosyl-9-methyl-sphingadienine) extract. This GR extraction method is highly efficient (>80%) and rapid compared to conventional extraction methods.

On the other hand glucosylceramides (GlcCers) are abundant in natural resources (~20 mol% of total lipids), whereas sphingoid bases are less abundant. And there is no practically efficient method to prepare sphingoid bases from GlcCers. In this study the author was developed a two-step chemoenzymatic method to prepare sphingoid bases from various kinds of GlcCers. The first step is performed by an alkali-catalyzed hydrolysis reaction accelerated by microwave irradiation leading selective and efficient cleave of the amide bond to obtain lysoGlcCers. The second step is accomplished by an enzymatic hydrolysis of the  $\beta$ -glycosidic bond in GlcCers by almond  $\beta$ -glucosidase for industrial use. The removal of the acyl chain will increase the hydrophilic character to increase accessibility toward its enzymatic active center of  $\beta$ -glucosidase. The current strategy is increase accessibility toward the enzyme by removing the hydrophobic fatty acid chain is quite unique and practical. Several sphingoid bases were successfully prepared in high yield by employing the designed method from a wide variety of dietary GlcCers. Additionally, the author performed preliminary biological screening of prepared sphingoid bases and their derivatives as multidrug resistance inhibitors. The initial result suggests that certain sphingoid bases are specifically cytotoxic to adriamycin resistant MCF-7 cells (breast cancer) rather than MCF-7 cells.

In conclusion, the author has new findings by discovering two novel methods for extraction/preparation of sphingoid bases, which are more simple, efficient, economical, and practically applicable for a large scale. GR based extraction technology is a promising tool for the discovery of new sphingoids-based chemotherapeutics. The established novel chemoenzymatic method is more efficient and economical for the preparation of sphingoid bases of natural origin. These research works will be more beneficial for industries, lipid chemists and biochemists, to explore more biological activities<sup>1</sup> of many unknown natural sphingoid bases. Therefore, we acknowledge that the author is qualified to be granted the Doctorate of Life Science from Hokkaido University.